MicroBiome Assay *Taq* DNA Polymerase

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I. Kit Content

Cat. No	MBA *Taq* DNA Polymerase	10X Reaction buffer	Dilution Buffer	dNTP Mixture	$MgCl_2$ Solution
E-3504	500 units	1 mL (without $MgCl_2$)	1 mL	1 mL	1 mL

II. Specifications

MicroBiome Assay *Taq* DNA Polymerase

Concentration	5 units/μl
5′→3′ exonuclease activity	Yes
3′→5′ exonuclease activity	No
3′ A overhang	Yes
Nuclease Contamination	No
Extension rate	3–8 kb/minute depending on template complexity

Buffer and Reagents

10X Reaction Buffer Without $MgCl_2$	: 200 mM Tris-HCl, 500mM KCl Tween 20 0.1%
Dilution Buffer	: 20mM Tris-HCl, 0.5mM EDTA, 1mM DTT, 100mM KCl 50% glycerol, pH 7.4, Stabilizer
dNTP Mixture	: 10mM (2.5mM each dNTP)
$MgCl_2$ Solution	: 20mM

III. Storage Conditions

MicroBiome Assay *Taq* DNA polymerase, including buffers and reagents, should be stored immediately upon receipt at -20℃.

If stored in the recommended temperature, this product will be stable until the expiration date printed out on the label.

IV. Applications

Routine PCR, dsDNA binding dye based qPCR, dual-labeled probe based qPCR, primer extension, TA cloning, microbial PCR Assay.

V. Description

Most *Taq* DNA Polymerases are highly likely to be contaminated with Host (*E.coli*) DNA. MBA (MicroBiome Assay) *Taq* DNA Polymerase is purified by a unique method that minimizes host DNA contamination and can be very useful for detecting microorganisms using 16S rRNA specific primers.

VI. Unit Definition

One unit is defined as the amount of enzyme of that incorporates 10 nmole of dNTP into acid-insoluble material in 30 minutes at 72℃.

VII. Protocol

1. Thaw 10X Reaction Buffer, dNTP mix, primer solutions and template DNA.
2. Prepare a Reaction mixture.

Component	20 μl reaction	50 μl reaction
Template	Variable	Variable
Forward primer (10 pmole/μl)	1 – 2 μl	2.5 - 5 μl
Reverse primer (10 pmole/μl)	1 – 2 μl	2.5 - 5 μl
10X Reaction Buffer	*2* μl	5 μl
10 mM dNTP (2.5 mM each)	2 μl	5 μl
MBA *Taq* DNA Polymerase (5 units/μl)	1 – 2 unit	1.25 – 2.5 unit
20 mM $MgCl_2$	1.5 μl	3.75 μl
PCR grade water	Variable	Variable

3. Mix the reaction mixture thoroughly and dispense appropriate volumes into PCR tubes.
4. Add Template DNA to individual PCR tubes.
5. Perform the reaction under the following conditions.
   For Standard PCR (3-step)

Step	Temperature	Time	Cycles
Pre-denaturation	94℃	1 min *	1 cycle
Denaturation	94℃	15-20 sec	25-35 cycles
Annealing	AT **	15-30 sec	
Extension	72℃	1 min/kb	
Final extension	72℃	Optional. Normally 3-5 min	1 cycle

*The Pre-denaturation step can be extended up to 5 minutes for genomic DNA.
** Annealing temperature (approximately 5℃ below Tm of primers).

6. Maintain the reaction at 4℃ after the completion of amplification. It is recommended to store samples at -20℃ until use.
   Analyze the PCR products by agarose Gel electrophoresis.

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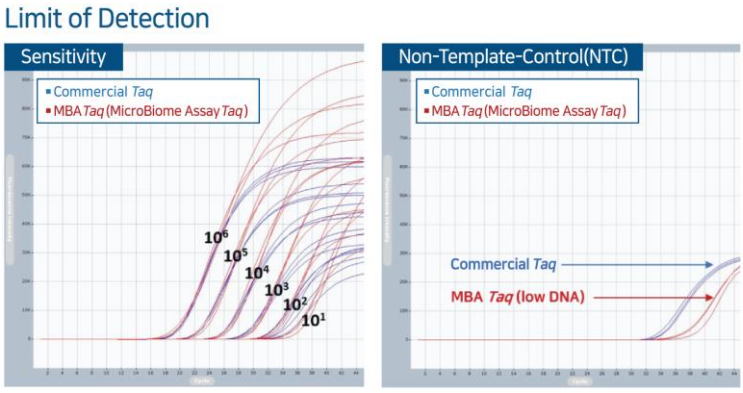
VIII. Experimental Data



	10^6 fg	10^5 fg	10^4 fg	10^3 fg	10^2 fg	10^1 fg	NTC
Commercial *Taq*	19.7	23.1	26.6	29.7	32.7	34.7	34.8
MBA *Taq*	19.8	23.2	26.8	30.1	33.0	35.2	38.8

Figure 1. Limit of Detection of MicroBiome Assay *Taq* DNA polymerase.
Compare *Taq* DNA polymerase (blue) with MicroBiome Assay *Taq* DNA Polymerase (Red). Limit of Detection and *E.coli* gDNA Contamination (45 cycle)

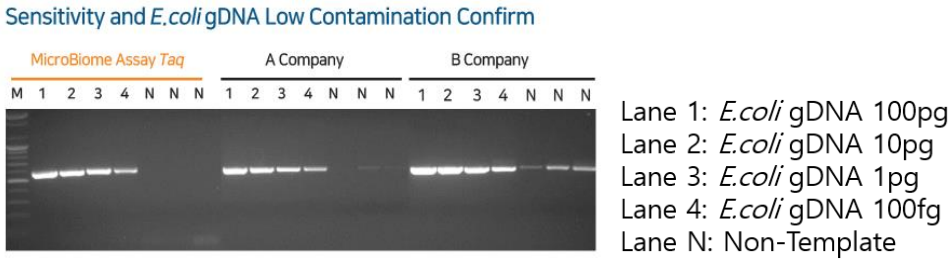


Figure 2. High sensitivity and Low Contamination DNA of MicroBiome Assay *Taq* DNA Polymerase.

IX. Notice

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